AD_____
(Leave blank)

Award Number: W81XWH-08-2-0171

TITLE: **Circadian Genes and Risk for Prostate Cancer**

PRINCIPAL INVESTIGATOR: Ann W. Hsing, PhD

CONTRACTING ORGANIZATION: T.R.U.E. Research Foundation
San Antonio, TX 78217

REPORT DATE: September 2009

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: (Check one)

☒ Approved for public release; distribution unlimited

☐ Distribution limited to U.S. Government agencies only; report contains proprietary information

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
09/03/2009	Annual	1 Sept 2008–31 Aug 2009

4. TITLE AND SUBTITLE

Circadian Genes and Risk for Prostate Cancer

5a. CONTRACT NUMBER
W81XWH-08-2-0171

5b. GRANT NUMBER
W81XWH-08-2-0171

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Ann W. Hsing, Ph.D.
Email: HsingA@mail.nih.gov

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

T.R.U.E. Research Foundation
Terri Nakamura
8610N New Braunsfel, Suite 705
San Antonio, TX 78217

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT:

We propose that genetic susceptibility to prostate cancer may be in part due to variations in the core circadian genes that regulate circadian rhythms and that serum sex steroid hormone levels modify the effect of circadian gene polymorphisms on prostate cancer risk. Our study is nested within the Prostate Cancer Prevention Trial (PCPT), a randomized placebo-controlled clinical trial to determine if finasteride (an inhibitor of androgen bioactivation) could prevent prostate cancer. In Year 1 of the award, we have maintained communications with the PCPT Statistical Center, been involved in subject selection for the study, and have been involved in analyzing serum androgen data that will be used for Aim 2 of our study. In addition, we have been working with the Roswell Park Cancer Institute Microarray and Genotyping Facility to develop and quality check the genotyping assays for our study. We have determined that our SNP set will include 311 SNPs and that at least two different platforms are necessary to genotype the entire SNP set. These tasks have been accomplished in line with the approved statement of work and ensure that we are within our timeframe of completing the study.

15. SUBJECT TERMS

Prostate cancer, circadian genes, genetic susceptibility

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	7	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

INTRODUCTION:

Prostate cancer is the most commonly diagnosed cancer and the second leading cause of cancer death in men in the United States yet the only established risk factors for prostate cancer are race, age, and family history [1]. Recent data from observational studies on sleep duration [2], light at night [3], rotating shift workers [4, 5], and male airline pilots [6-8] suggest that circadian rhythm disruptions increase prostate cancer risk; no underlying molecular mechanism has yet been identified. We propose that genetic susceptibility to prostate cancer may be in part due to variations in genes from a number of pathways including the core circadian genes that regulate circadian rhythms. The goal of this project is to test the novel hypothesis that variants in circadian genes alter the risk of prostate cancer and that serum sex steroid hormone levels modify the effect of circadian polymorphisms on prostate cancer risk. Our study is nested within the Prostate Cancer Prevention Trial (PCPT), a randomized placebo-controlled clinical trial to determine if finasteride (an inhibitor of androgen bioactivation) could prevent prostate cancer. Included in our study are approximately 1,800 case-control pairs (3,600 individuals), for which several biological measurements are available, including serum sex hormone levels, which we will also incorporate into our study to test our hypothesis.

BODY:

Our study has three specific aims, all of which utilizes genotyping data that is to be generated in Award Years 1-2. The first year of the award involved four tasks as outlined in the Statement of Work. The following is a report as it pertains to each task in Award Year 1:

Task 1 Data management
Performance sites: NCI and PCPT Statistical Center
Performance period: Months 1-36 (entire period)

We have been in constant communications with the PCPT Statistical Center at the Fred Hutchinson Cancer Research Center (Seattle, WA) since the project's inception. Genotyping assays that will generate data for this project is underway and the resulting data will be incorporated into the PCPT central database when available. We have also been working with the PCPT Statistical Center on analyzing data related to serum androgen levels as part of the PCPT Program Project, in which we have nested the current study. The androgen data will be incorporated into the current study as part of Aim 2.

Task 2 Develop and perform genotyping assays on 320 SNPs (including 40 putatively functional and 270 tag SNPs as well as additional SNPs to account for control SNPs and potential SNP assay failures) of circadian genes in approximately 4,000 samples including 1,800 case-control pairs (3,600 subjects) and approximately 400 duplicate quality control samples.
Performance sites: Roswell Park Cancer Institute (RPCI) Microarray and Genomics Facility.

Method: Multiplex genotyping using established protocols including MassARRAY Analyzer Compact system (Sequenom)
Performance period: Months 1-24
Anticipated Outcome: Genotyping results on approximately 320 SNPs for 3,600 subjects.

We have been working with RPCI Microarray and Genomics Facility to develop the genotyping assays for the study. First, we decided to change the genotyping platform from the Sequenom MassARRAY to the Illumina Golden Gate Multiplex Genotyping Platform. This genotyping platform is superior to the originally proposed Sequenom MassARRAY system because it is able to interrogate 386 SNPs (as opposed to ~20 SNPs) in one assay using less DNA, and the assay design is more translatable to other Illumina genotyping platforms if alternative platforms are necessary to complete the genotyping. This change in protocol does not change the aims or outcomes of the project. Second, we designed, developed, and quality control-tested the SNPs to be assayed for the project. From the 321 SNPs identified for the project, 10 SNPs failed assay design, 13 SNPs need alternative genotyping platforms, and 298 SNPs were able to be accommodated on the Illumina Golden Gate Multiplex Genotyping Platform. Thus, the final SNP set will be 311 SNPs with 298 SNPs to be genotyped on the Illumina Golden Gate platform and 13 SNPs to be genotyped using alternative (uniplex) platforms. Genotyping assays will begin in the early part of Award Year 2 as planned.

Task 3 Monitor quality of genotyping results on an ongoing basis
Performance sites: NCI, PCPT Statistical Center
Method: Analyses of Hardy-Weinberg equilibrium, inter-individual genotype call rates, concordance of duplicate specimens or known controls, and other quality control (QC) measures.
Performance period: Months 7-36

As mentioned for Task 2, we have been developing the genotyping assay with RPCI. The initial assay development included an initial quality control test to determine if primers designs for the SNPs are within the limitations of the multiplex genotyping platform; this include ensuring that melting temperatures for the primers are similar enough to each other for all SNPs in the multiplex genotyping set. As noted above, 10 SNPs did not survive the assay development and have been removed from the SNP set. Thirteen SNPs required alternative genotyping platforms because the melting temperatures for the primers were too different than the other 298 SNPs. Thus, for the Illumina Golden Gate Multiplex Platform, 298 of the 311 SNPs in our SNP set will be included. Once genotyping begins for the PCPT specimens, we will continue to monitor genotyping quality including inter-individual genotype call rates, concordance of duplicate specimens, among other measures.

Task 4 Gather, ship, process, and archive biospecimens
Performance sites: PCPT Biorepository in Colorado and Roswell Park Cancer Institute (RPCI) Microarray and Genomics Facility.
Performance period: Months 1-20

We have been working with PCPT Statistical Center and other PCPT Program Project investigators on determining the final set of subjects to be included in the study. Our initial estimate included 1,800 case-control pairs (3,600 subjects total) that were included in the PCPT Program Project; all of these subjects have serum androgen measurements. Of these 3,600 subjects, DNA from about 2,800 subjects are ready for genotyping. The remaining 800 subjects have insufficient DNA for genotyping and efforts led by the PCPT Program Project are underway to collect additional samples from the participants and to determine if serum-derived DNA may be used for multiplex genotyping; currently, methods to isolate DNA from serum have been tested for uniplex genotyping, which is not feasible for genotyping a SNP set of 311 SNPs due to limitations in amount of DNA obtainable from serum as well as costs associated with the uniplex assays.

KEY RESEARCH ACCOMPLISHMENTS:

- Changed the genotyping platform to the Illumina Golden Gate Multiplex Platform, which is able to interrogate 384 SNPs in one assay as compared to the ~20 using the Sequenom MassARRAY system as originally proposed.
- Designed and quality checked SNPs identified for the project. The final SNP set (311 SNPs) is to include 298 SNPs to be genotyped on the Illumina Golden Gate Multiplex Genotyping Platform and 13 SNPs to be genotyped by alternative platforms.
- Identified about 2,800 subjects that have sufficient DNA for all genotyping assays for the study; alternative methods for obtaining sufficient DNA for the remaining 800 subjects of the PCPT nested case-control study led by the PCPT Program Project are underway.

REPORTABLE OUTCOMES:

There are currently no reportable outcomes from this project.

CONCLUSION:

The goal of this project is to test the novel hypothesis that variants in circadian genes alter the risk of prostate cancer and that serum sex steroid hormone levels modify the effect of circadian polymorphisms on prostate cancer risk. In Year 1 of the award, we have maintained communications with the PCPT Statistical Center, been involved in subject selection for the study, and have been involved in analyzing androgen data that will be used for Aim 2 of our study. In addition, we have been working with RPCI to develop and quality check the genotyping assays for our study. We determined that the SNP set for the study will include 311 SNPs and that at least two different platforms are necessary to genotype the entire SNP set. These tasks have been accomplished in line with the approved statement of work and ensure we are within our timeframe of completing the study.

REFERENCES:

1 Hsing AW, Chokkalingam AP. Prostate cancer epidemiology. Front Biosci 2006;11:1388-413.
2 Kakizaki M, Inoue K, Kuriyama S, et al. Sleep duration and the risk of prostate cancer: the Ohsaki Cohort Study. Br J Cancer 2008;99:176-8.
3 Kloog I, Haim A, Stevens RG, et al. Global Co-Distribution of Light at Night (LAN) and Cancers of Prostate, Colon, and Lung in Men. Chronobiol Int 2009;26:108 - 25.
4 Kubo T, Ozasa K, Mikami K, et al. Prospective cohort study of the risk of prostate cancer among rotating-shift workers: findings from the Japan Collaborative Cohort Study. Am J Epidemiol 2006;164:549-55.
5 Conlon M, Lightfoot N, Kreiger N. Rotating shift work and risk of prostate cancer. Epidemiology 2007;18:182-3.
6 Band PR, Le ND, Fang R, et al. Cohort study of Air Canada pilots: mortality, cancer incidence, and leukemia risk. Am J Epidemiol 1996;143:137-43.
7 Irvine D, Davies DM. British Airways flightdeck mortality study, 1950-1992. Aviat Space Environ Med 1999;70:548-55.
8 Pukkala E, Aspholm R, Auvinen A, et al. Cancer incidence among 10,211 airline pilots: a Nordic study. Aviation, space, and environmental medicine 2003;74:699-706.

APPENDICES:

None

SUPPORTING DATA:

None.